TABLE 1. Production of nitric oxide and tumoricidal properties in mouse macrophages by liposomes containing MTP-PE, CGP31362 and JT3002

Concentration		MI V-HBSS	_	MLV-MTP-PE		MLV-31362		MLV-JT3002
of MLV (nmol/well)	NO (MH)		NO (MM)	NO Cytotoxocity (μM) (%)	NO (Μπ)	NO Cytotoxicity (μΜ) (%)	NO (µM)	NO Cytotoxicity (μM) (%)
50	ω	4	4	19	28,	84*	30,	86*
25	ស	0	2	14	26,	74*	29,	80,
12	-	 -	2	10	23*	79*	23,	84*
9	1	2	2	ഹ	22*	72*	22,	70,
т	-	0	2	· 4	20•	75,	22,	68*

IFN- γ . All MLV contained 1 mg immunomodulator/300 μ M phospholipids. NO production (nitrite/nitrate) was determined one day later. The cultures were washed and 1 x 10' ['H]TdR-labeled A375P cells were added. Assays were terminated 72 h later. Macrophages incubated in medium alone (negative control) produced 0.2 μM NO and 10% cytotoxicity. Macrophages in medium containing LPS (1 μ g/ml) and IFN- γ (10 U/ml) produced 26 μ M NO and 48% cytotoxicity (P<0.001). The values are the mean of triplicate cultures. Variation from the mean did not exceed Macrophages (1 x 10³/well) were incubated with the indicated concentrations of MLV in medium containing 10 U/ml 10%. These are the results of one representative experiment of four.

.p<0.001

TABLE 2. Minimal concentration of liposome-JT3002 required to induce production of nitric oxide in murine macrophages

Lipid		ΝΟ (μ	1	
concentration (nmol/well)	JT3002 (0.1 mg)	JT3002 (0.02 mg)	JT3002 (0.004 mg)	JT3002 (0.0008 mg)
25	27*	23*	10*	11
12.5	26*	20°	14*	9
6.2	24*	17*	12*	7
3.1	24*	16 ²	10	7
1.6	21*	13*	9	7
0.8	17*	11	9	7
0.4	19 *	11	10	7
0.2	18*	10	10	6

Macrophages (1 x 10 $^{\circ}$ /well) were incubated in medium containing 10 U/ml IFN- γ (control) or medium containing 10 U/ml IFN- γ and different concentrations of liposomes containing 0.1 mg, 0.02 mg, 0.004 mg, or 0.008 mg JT3002 in 300 μ M phospholipids. NO production was determined 24 h later. The values are the mean NO proudction in μ M of triplicate cultures. Variation from the mean did not exceed 10%. Macrophages incubated with medium plus IFN- γ or medium containing IFN- γ plus LPS produced 9 and 25 μ M NO, respectively. This is one representative experiment of three.

*P<0.001.

TABLE 3. Activation of tumoricidal properties in macrophages from iNOS knockout mice

Lipid	·	NO (MM)			Cytotoxicity (%)	(%)
(nmol/well)	+/+ mice	+/- mice	-/- mice	+/+ mice	+/- mice	-/- mice
20	21*	14°	0	93*	91*	7
25	20•	14°	0	93*	89 _*	1.5
10	17*	12	0	85,	.29	0
S.	16	11	0	31*	51*	0
0	0.	0	0	0	0	0
LPS (1 µg/ml)	204	13	0			

or CT-26 (not shown) cells were added. NO production ($\mu M/10^3$ macrophages) was determined after 20 h and $\mu g/ml$ LPS (positive control), or medium containing different concentrations of MLV containing 0.1 mg 3T3002/300cytotoxicity was determined after 72 h. The values are the mean of triplicate samples. Variation from the mean Macrophages (1 x 10'/well) were incubated in medium containing 10 U/ml IFN-γ (control) or medium containing l μ M phospholipid. After 20 h incubation, the cultures were washed and 1 x 10 $^{\circ}$ [3 H]TdR-labeled K-1735 M2 (shown) did not exceed 15%. This is one representative experiment of three.

*P<0.01.

^bP<0.05.

TABLE 4. Activation of tumoricidal properties in macrophages from LPS-responsive (C3H/HeN) and -nonresponsive (C3H/HeJ) mice

concentration	<u>C</u>	3H/HeN mice	C3	H/HeJ mice
(nmol/well)	NO (μM)	Cytotoxicity (%)	NO (μM)	Cytotoxicity (%)
20	23*	35*	32,	40*
2	11	28°	26*	32*
0.2	2	13	13	27*
0.02	5	7	9	11
0	2	3	0	6
LPS (1 μg/ml)	23*	36 °	. 8	12

Macrophages (1 x 10 5 /well) were incubated in medium containing 10 U/ml IFN- γ (control), or medium containing 1 μ g/ml LPS (positive control), or medium containing different concentrations of MLV containing 0.1 mg JT3002/300 μ M phospholipid. After 20 h incubation, the cultures were washed and 1 x 10 4 [3 H]TdR-labeled K-1735 M2 cells were added. NO production (nitrite) was determined after 20 h and cytotoxicity was determined after 72 h. The values are the mean of triplicate samples. Variation from the mean did not exceed 10%. This is one representative experiment of three.

*P<0.01.

TABLE 5. Duration of tumoricidal activity in macrophages incubated with liposomes containing JT3002

Days post-	NO (<u>ι</u> Μ)	Cytot	oxicity (%)
activation	Medium	JT3002	Medium	JT3002
1	0.9	31.8	5.9	49.7
2	1.3	34.0	6.6	19.8
3	0.7	27.7	4.1	19.2
4	4.9	4.0	5.9	4.8
Reactivation				
5	2.2	33.7	3.0	41.0

Macrophages (1 x 10 3 /well) were incubated in medium containing 10 U/ml IFN- γ (control) or medium containing 10 U/ml IFN- γ plus 1 nmol/well of MLV containing 0.1 mg JT3002/300 μ M phospholipid. After 20 h incubation, the cultures were washed and fresh medium was added for 0, 1, 2, 3, or 4 days. At the different time points, 1 x 10 4 [3 H]TdR-labeled CT-26 cells were added. NO production (nitrite/nitrate) was determined at the indicated times. Cytotoxicity was determined after 72 h of continuous tumor-cell-macrophage interaction. The values are the mean of triplicate cultures. Variation from the mean did not exceed 10%. This is one representative experiment of two.

^{*}P<0.001.

P<0.01.

TABLE 7
Combination Therapy of MTP-PE and CPT-11 for Mouse
CT-26 Colon Cancer Liver Metastasis

Oral			pleen	Live	er
treatment	CPT-11	Weight (g)	Tumor size (mm)	Weight	Median no metastases
Saline	Saline	1.5 ± 0.1	1.4 ± 0.7	7.4 ±1.6	>100
Saline	50 mg/kg	0.6 ± 0.2	8.3 ± 2.0	2.0 ± 0.3	30
Saline	100 mg/kg		All mic	e died	·
MTP-PE	50 mg/kg	0.6 ± 0.2	10.4 ± 2	2.2 ± 0.7	30
MTP-PE	100 mg/kg	0.3 ± 0.1	5.6 ± 2	1.2 ± 0.1	4

Table 19. Therapy of experimental liver metastasis produced by murine CT-26 colon carcinoma with CPT-11 in combination

with either MLV-JBT 3002 or free-form (FF) JBT 3002

		Sple	Spicen (primary)		Liver (metastasis)	
	∆BW°	Incidence	Tumor volume	Incidence	Median (range)	Liver weight
Treatment	(%)		(mm)			(g)
MLV-HBSS	6.4	5/5	567 ± 94	5/5	46, 56, 72, >100, >100 3.5 ± 1.6	3.5 ± 1.6
MLV-HBSS + CPT-11	-1.7	2/5	140 ± 30'	2/2	12, 15, 18, 39, 73	1.8 ± 0.3"
MLV-JBT3002 (1.0µg/dose) + CPT-11	-0.4	5/5	56±29°	2/5	0, 0, 0, 6, 12	1.6 ± 0.2^{b}
MLV-JBT3002 (0.1 µg/dose) + CPT-11	-0.8	5/5	72 ± 15°	3/5	0, 0, 4, 8, 79	1.6 ± 0.2^{b}
FF-JBT3002 (1.0µg/dose) + CPT-11	-3.9	5/5	202 ± 69 ^b	2/5	7, 25, 37, 53, 81	1.8 ± 0.4^{b}
FF-JBT3002 (0.1µg/dose) + CPT-11	0	5/5	85±23°	3/5	0, 0, 9, 13, 35	1.5 ± 0.3^{b}

Five BALB/c mice per group were given intrasplenic injection of 1 x 10° CT-26 cells on day 0. Mice were treated with repeated oral feedings of MLV-JBT3002 (at either 1.0 or 0.1 µg/dose, 5µmol PCPS MLV), or FF-JBT3002 (at either 1.0 or 0.1 µg/dose) thrice weekly for 3 weeks beginning 3 days after tumor cell inoculation, in combination with 100 mg/kg CPT-11 i.p. once a week (on day 7, 14, and 21). All groups were killed on day 23.

	27	- 5		
	21	√ -	: 	CF1-11
	17 18 19	4 4 4	JBT3002	
	# .	 		CPT-11
days	10 11 12	4 4 4	JBT3002	
	7	4	_	CPT-11
	3 + 5	4 4 4	JBT3002	J
	0-		CT-26	

The rate of body weight reduction was calculated with the formula: $\Delta BW(\%) = (AB-1) \times 100$, where A = mean body weights of mice at death, and B = Pmean body weights of mice on day 0.

 $^{^{}b}P < 0.05, ^{c}P < 0.005,$ compared with MLV-HBSS

with either MLV-JBT 3002 or free-form (FF) JBT 3002

		Spl	Spleen (primary)		Liver (inclastasis)	
	ΔΒΝ" (%)	Incidence	Tumor volume (mm³)	Incidence	Median (range)	Liver weight (g)
Irealinent	2.4	5/5	701 ± 268	5/5	54, >100, >100, >100, >100 4.2 ± 1.2	4.2 ± 1.2
MLV-HBSS + saline	i -	5/5	189 ± 71°	5/5	22, 24, 39, 47, 57	1.7 ± 0.3°
CPT-11		5/5	154 ± 136"	3/5	0, 0, 3, 4, 13	1.4 ± 0.1
MLV-JBT3002 (1.0\u00f46se) + Crifi		2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2	238 ± 70 ⁶	5/5	5, 27, 31, 53, 80	1.7 ± 0.4°
FF-JBT3002 (1.0µg/dose) + CF1-11	1.7	5/5	290 ± 106 ^b	5/5	1, 3, 10, 14, 34	1.5 ± 0.5
FF-JB 13002 (0.1µg/dose) + CPT-11	-1.0	5/5	181 ± 115°	4/5	0, 1, 3, 14, 32	1.4 ± 0.4°

BALBic mice were given intrasplente injection of 1 x 10⁴ CT-26 cells on day 0. Mice were treated with oral feedings of MLV-JBT3002 (at either 1.0 or 0.1 µg/dose, 5µmol PCPS MLV), or FF-JBT3002 (at either 1.0 or 0.1 µg/dose) thrice weekly for 3 weeks beginning 3 days after tumor cell inoculation, in combination with 100 mg/kg CPT-11 i.p. once a week (on day 7, 14, and 21). All groups were killed on day 24.

	7.7	Kill -
	12	 CPT-111
	61 81 21	4 4 4 JBT3002
	<u> </u>	CPT-111
days	10 11 12	4 4 4 JBT3002
	7	CPT-11
	3 4 5	4 4 4 JBT3002
	0	CT-26

"Changes in body weight were calculated by the formula: $\Delta BW'(\vec{e_b}) = (A + B) B \times 100$, where A = mean body weight of mice at death, and B = mean body

weight of mice on day 0.

 $P < 0.05, \, ^{\circ}P < 0.005, \, {\rm compared \ with \ MLV-HBSS} + {\rm saline}$

Therapy of experimental liver metastasis produced by murine C1-20 colou calcinolia minima minima more --Table 12.

injections in combination with either MLV-JBT 3002 or free-form (FF) JBT 3002 at different doses

		Sple	Spleen (primary)		Liver (metastasis)	
	ABW"	Incidence	Tumor volume	Incidence	no.	Liver weight
Treatment	(%)		(mm)			(2)
MLV-HBSS + saline	5.1	5/5	153 ± 62	5/5	23, 26, 71, >100, >100	2.4 ± 1.0
MLV-HBSS + CPT-11	-17.6	5/5	52 ± 30	2/5	0, 0, 0, 1, 6	1.2 ± 0.1
MLV-JBT3002 (1.0μg/dose) + CPT-11	-1.5	5/5	45±10	0/5	all 0	1.4 ± 0.1
FF-JBT3002 (1.0µg/dose) + CPT-11	-2.4	5/5	48 ± 8	2/5	0,0,0,3,5	1.4 ± 0.03
FF-JBT3002 (0.1µg/dose) + CPT-11	-2.2	5/5	50 ± 16	1/5	0,0,0,0,3	1.4 ± 0.2
FF-JBT3002 (0.01 μ g/dose) + CPT-11	9.0	. 5/5	29 ± 26	4/5	0, 2, 2, 26, 27	1.6 ± 0.1
FF-JBT3002 (0.001 μ g/dose) + CPT-11	-6.9	5/5	56 ± 25	1/5	0,0,0,0,3	1.4 ± 0.2
FF-JBT3002 (0.0001μg/dose) + CPT-11	-15.4	5/5	28 ± 20	3/5	0, 0, 1, 2, 5	1.1 ± 0.1

MLV-JBT3002 (1 µg/dose), or FF-JBT3002 (at either 1.0, 0.1, 0.001, or 0.0001 µg/dose) for 3 consecutive days beginning 3 days after rumor cell BALB/c mice were injected into the spleen with 1 x 10⁴ viable CT-26 cells on day 0. Mice were treated with oral feedings of 5 µmol MLV-HBSS, inoculation. Seven days later, groups of mice received 4 daily i.p. injections of 100 mg/kg CPT-11. All groups were killed on day 14.



*Changes in body weight were calculated by the formula: $\Delta BW(\%) = (A \cdot B) B \times 100$, where A = mean body weight of mice at death, and B = mean body

weight of mice on day 0.

Table 13. Therapy of experimental liver metastasis produced by murine CT-26 colon carcinomas with once weekly CPT-11 injections in combination with either MLV-JBT 3002 or free-form (FF) JBT 3002 at different doses

ΔBW° Incidence Tumor volume Incidence MLV-HBSS + saline 3.1 5/5 699 ± 322 5/5 MLV-HBSS + cPT-11 1.2 5/5 334 ± 88 5/5 MLV-HBSS + CPT-11 1.3 5/5 157 ± 96 4/5 MLV-JBT3002 (1.0µg/dose) + CPT-11 1.4 5/5 235 ± 78 5/5 FF-JBT3002 (0.0µg/dose) + CPT-11 -0.2 5/5 189 ± 13 5/5 FF-JBT3002 (0.01µg/dose) + CPT-11 0.3 5/5 214 ± 45 5/5 FF-JBT3002 (0.01µg/dose) + CPT-11 2.5 5/5 237 ± 20 5/5 FF-JBT3002 (0.01µg/dose) + CPT-11 2.5 5/5 237 ± 20 5/5			Sple	Spleen (primary)		Liver (metastasis)	
Treatment (%) (mm³) MLV-HBSS + saline 3.1 5/5 699 ± 322 5/5 MLV-HBSS + CPT-11 1.2 5/5 334 ± 88 5/5 MLV-JBT3002 (1.0 μg/dose) + CPT-11 1.3 5/5 157 ± 96 4/5 FF-JBT3002 (1.0 μg/dose) + CPT-11 -1.4 5/5 23,5 ± 78 5/5 FF-JBT3002 (0.1 μg/dose) + CPT-11 -0.2 5/5 189 ± 13 5/5 FF-JBT3002 (0.01 μg/dose) + CPT-11 0.3 5/5 214 ± 45 5/5 FF-JBT3002 (0.001 μg/dose) + CPT-11 2.5 5/5 237 ± 20 5/5		ABW"	Incidence	Tumor volume	Incidence	no.	Liver weight
3.1 5/5 699±322 5/5 1.2 5/5 334±88 5/5 1.3 5/5 157±96 4/5 -1.4 5/5 235±78 5/5 -0.2 5/5 189±13 5/5 0.3 5/5 214±45 5/5 2.5 5/5 237±20 5/5	Treatment	(%)		(mnn ³)			(g)
MLV-HBSS + CPT-11 MLV-JBT3002 (1.0μg/dose) + CPT-11 FF-JBT3002 (0.01μg/dose) + CPT-11 FF-JBT3002 (0.01μg/dose) + CPT-11 FF-JBT3002 (0.01μg/dose) + CPT-11 FF-JBT3002 (0.001μg/dose) + CPT-11	MLV-HBSS + saline	3.1	5/5	699 ± 322	5/5	89, >100, >100, >100, >100 4.1 ± 0.8	4.1 ± 0.8
MLV-JBT3002 (1.0μg/dose) + CPT-11 1.3 5/5 157 ± 96 4/5 FF-JBT3002 (1.0μg/dose) + CPT-11 -1.4 5/5 235 ± 78 5/5 FF-JBT3002 (0.1μg/dose) + CPT-11 -0.2 5/5 189 ± 13 5/5 FF-JBT3002 (0.01μg/dose) + CPT-11 0.3 5/5 214 ± 45 5/5 FF-JBT3002 (0.001μg/dose) + CPT-11 2.5 5/5 237 ± 20 5/5 FF-JBT3002 (0.001μg/dose) + CPT-11 2.5 5/5 237 ± 20 5/5 5/5 FF-JBT3002 (0.001μg/dose) + CPT-11 2.5 5/5 237 ± 20 5/5 5/5 5/5 5/5 5/5 5/5 5/5 5/5 5/5 5/	MLV-HBSS + CPT-11	1.2	5/5	3,34 ± 88	515	42, 42, 45, 56, 79	2.6 ± 0.3
FF-JBT3002 (1.0μg/dose) + CPT-11 -1.4 5/5 235 ± 78 5/5 FF-JBT3002 (0.1μg/dose) + CPT-11 -0.2 5/5 189 ± 13 5/5 FF-JBT3002 (0.01μg/dose) + CPT-11 0.3 5/5 214 ± 45 5/5 FF-JBT3002 (0.001μg/dose) + CPT-11 2.5 5/5 237 ± 20 5/5 5/5	MLV-JBT3002 (1.0μg/dose) + CPT-11	1.3	5/5	157 ± 96	4/5	0, 1, 9, 11, 13	1.5 ± 0.2
FF-JBT3002 (0.1μg/dose) + CPT-11 -0.2 5/5 189 ± 13 5/5 FF-JBT3002 (0.01μg/dose) + CPT-11 0.3 5/5 214 ± 45 5/5 FF-JBT3002 (0.001μg/dose) + CPT-11 2.5 5/5 237 ± 20 5/5 FF-JBT3002 (0.001μg/dose) + CPT-11 2.5 5/5 237 ± 20 5/5	FF-JBT3002 (1.0µg/dose) + CPT-11	-1.4	5/5	23,5 ± 78	2/5	34, 41, 56, 70, 88	2.6 ± 0.6
FF-JBT3002 (0.01μg/dose) + CPT-11 0.3 5/5 214±45 5/5 FF-JBT3002 (0.001μg/dose) + CPT-11 2.5 5/5 237±20 5/5 5/5	FF-JBT3002 (0.1µg/dose) + CPT-11	-0.2	5/5	189 ± 13	5/5	3, 12, 16, 24, 34	1.6 ± 0.4
FF-JBT3002 (0.001 μ g/dose) + CPT-11 2.5 5/5 237 ± 20 5/5	FF-JBT3002 (0.01μg/dose) + CPT-11	0.3	5/5	214 ± 45	5/5	2, 4, 13, 31, 40	1.6 ± 0.3
5/5 10 312 00 11 110	FF-JBT3002 (0.001μg/dose) + CPT-11	2.5	5/5	237 ± 20	2/5	31, 42, 47, 58, 69	2.8 ± 0.7
FF-JBT3002 (0.0001μg/dose) + CPI-11 2.3 2.3 ± 34		2.3	2/5	225 ± 34	5/5	30, 32, 48, 52, 83	2.7 ± 0.9

MLV-HBSS, MLV-JBT3002 (1 µg/dosc), or FF-JBT3002 (at cither 1.0, 0.1, 0.001, or 0.0001 µg/dosc) thrice weekly for 3 weeks beginning 3 days after BALB/c mice were injected into the spleen with 1 x 104 viable CT-26 cells on day 0. Groups of mice were treated with oral feedings of 5 µmol tumor cell inoculation. Some mice received an i.p. injection of 100 mg/kg CPT-11 once a week (on days 7, 14, and 21). All groups were killed on day 23.

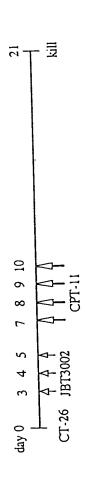
27	Kii -
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7	
10 11 12	4 4 4 JBT3002
7	CPT-11
3 4 5	4 4 4 JBT3002
day 0	CT-26

"Changes in body weight were calculated by the formula: $\Delta BW(\%) = (A \cdot B)Bx100$, where A = mean body weight of mice at death, and B = mean body weight of mice on day 0.

Table 14. Therapy of experimental liver metastasis produced by murine CT-26 colon carcinomas with intensive CPT-11 injections in combination with either MLV-JBT 3002 or free-form (FF) JBT 3002 at different doses

			Spleer	Spleen tumor		Liver metastasis	
	ΔBW14°	ΔBW21°	Incidence	ΔΒ\V ₁₄ ΔΒ\V ₂₁ Incidence Mean tumor	Incidence	No.	Liver weight
Treatment	(%)	(%)		volume (mm³)			(6)
Control	2.9	6.9	5/5	5/5 353 ± 29	2/5	5/5 54,>100,>100,>100,>100 3.4±1.1	3.4 ± 1.1
כסוויס	-24.0	Ν Ω	2/S	35 ± 16	_q S/0	all 0	1.2 ± 0.2
VI V IRT 3002 (1 0) 119/dose) + CPT-11	-9.4	-7.6	5/5	75 ± 64	3/5	0, 0, 3, 5, 16	1.5 ± 0.1
FE-JBT 3002 (0.05 µg/dose) + CPT-11	-6.8	-6.0	5/5	83 ± 70	4/5	0, 1, 9, 18, 21	1.7 ± 0.0

µg/dose), or FF-JBT 3002 (0.05 µg/dose) for 3 consecutive days beginning 3 days after turnor cell inoculation. Seven days later, groups of mice received 4 BALB/c mice were injected into the spleen with 1 x 10⁴ viable CT-26 cells on day 0. Mice were treated with oral feedings of 5 µmol MLV-JBT 3002 (1 daily i.p. injections of 100 mg/kg CPT-11. All groups were killed on day 21.



"Changes in body weight were calculated by the formula: $\Delta BW(\mathcal{G}_0) = (A - B)B \times 100$, where A = mean body weight of mice on the indicated day, and B

= mean body weight of mice on day 0.

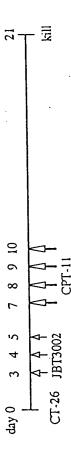
ND, not determined.

^bAll mice died during therapy (3 mice on day 15 and 2 mice on day 16)

injections in combination with oral JBT 3002

	Splee	Spleen tumor	-	Liver metastasis		
	Incidence	Mean tumor	Incidence	No.	A	Liver weight
Treatment		volume (mm³)				(B)
Control	10/10	594±51	10/10	85, >100, >100, >100, >100		3.2 ± 0.9
				>100,>100,>100,>100,>100		
CPT-11	6/10 ⁶	79 ± 38 c.	1/10	0, 0, 0, 0, 0, 0, 0, 0, 26	<0.0001	1.9 ± 0.3 cd
JBT 3002	10/10	88 ± 34′	9/10	0, 1, 2, 6, 10, 10, 11, 15, 22, 31	<0.0001	$1.6 \pm 0.2^{\circ}$
JBT 3002 + CPT-11	4/10	47 ± 26'	4/10	0, 0, 0, 0, 0, 0, 2, 5, 5, 8	<0.0001	1.4 ± 0.1^{f}

BALB:c mice were injected into the spleen with 1 x 10⁴ viable CT-26 cells on day 0. Groups of mice were treated with oral feedings of JBT 3002 (0.05 µg dosc) for 3 consecutive days beginning 3 days after tumor cell inoculation. Seven days later, some mice received 4 daily i.p. injections of 100 mg/kg CPT-11. All groups were killed on day 21.



[&]quot;. As compared with control.

^bSeven mice died during therapy (day 10, 13, 13, 14, 14, 17, 20).

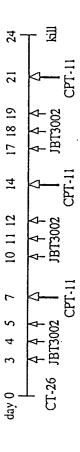
[&]quot;Calculated from survive mice.

[&]quot;P<0.05 as compared with control. "P<0.001 as compared with control. $^{f}P<0.0001$ as compared with control.

Table 14. Therapy of experimental liver metastasis produced by murine CT-26 colon carcinoma with once weekly CPT-11 injections in combination with oral JBT 3002

	Sple	Spleen tumor	V.	Liver metastasis		
	Incidence	Mean tumor	Incidence	No.	P	Liver weight
Treatment		volume (mm³)				(8)
Control	10/10	574 ± 101	10/10	72, >100, >100, >100, >100		4.3 ± 1.0
				>100, >100, >100, >100, >100		
CPT-11	7/10	116±32 ^b	8/10	0, 0, 1, 5, 6, 13, 33, 81, 85, >100	0.0005	2.0 ± 0.9°
JBT 3002	8/10	241 ± 84	01/6	1, 2, 50, >100, >100, >100		4.2 ± 1.6
				>100, >100, >100, >100, >100		
JBT 3002 + CPT-11	6/10	76 ± 34^{b}	2/10	0, 0, 0, 0, 0, 1, 6, 7, 37, 57	<0.0001	$1.7 \pm 0.4^{\circ}$

BALB c mice were injected into the spleen with 1 x 104 viable CT-26 cells on day 0. Groups of mice were treated with oral feedings of JBT3002 (0.05 ug dose) thrice weekly for 3 weeks beginning 3 days after tumor cell inoculation. Some mice received an i.p. injection of 100 mg/kg CPT-11 once a week (on days 7, 14, and 21). All groups were killed on day 24.



As compared with control.

 $^{^{}b}P<0.05$ as compared with control. $^{c}P<0.0001$ as compared with control.

Table 17. Induction of NO production in macrophages by free-form, formula 1, and formula 2 JBT 3002

- 1. Macrophages: TG-Mø from C57BL/6 mice.
- 2. Treatment of macrophages: Macrophages in 96-well plates (10⁵/well) were incubated for 24 hr with JBT in the presence or absence of IFN-γ (10 U/ml). Nitrite in the culture medium was then determined.
- 3. Results:

JBT conc. (ng/ml)	Free JBT		Formula (pH	a-1 JBT 1.5-7)		a 2-JBT 8)
	medium	IFN-g	medium	IFN-g .	medium	IFN-g
10	8.4	60.9*	2	50.7	2	47.4
2	0	53.1	0	38.6	0	38.
0.4	0	44.7	0	34.8		33.5
0.08	0	41	0	25.5	0	20
0.016	0	33.7	0	6.3		1.9
0.003	0	17.5	0	0.4	0	
0.0006	n.d.	n.d.	0	0.5	0	0.7
0	0	0.6		0.5	<u>U</u>	2

nitrite: μM.

LAL endotoxin test:

No endotoxin was detected in the free form JBT3002, Formula 1-JBT, and Formula 2-JBT at a concentration of 0.08 ng/ml of the reagent.

Table 19. Induction of NO production by JBT 3002.

1. Materials and Methods

- 1) Macrophages: C57BL/6 mice, TG-Mø, 10⁵ cells/well in 96-well plate.
- 2) Treatment: with 10 U/ml of IFN-y and various concentrations of JBT3002 for 24 hr in 200 μl/well MEM-5% FBS. Nitrite (100 μl/well) was measured.

2. Results				—— T.	ABUETS	
JBT3002 (ng/ml)	Free	form	•	filtered		iltered
	Medium 1	FN-γ	medium	IFN-γ	medium	IFN-γ
10 1 0.1 0.01 0.001 0.0001	0.5 0 0 0 0 0	47.1 37.7 27.7 19.5 8.5 0	0 0 0 0 0	41.0 29.3 20.9 7.7 0	7.0 0 0 0 0 n.d.	53. 0 44.5 34.1 26.2 4.3 n.d.

3. Endotoxin Test:

Endotoxin was not detected by the LAL assay in all of the three preparations of JBT3002 at concentration of 0.1 ng/ml.

4. CONCLUSION:

The contents in the tablet formulation did not alter the activity of JBT3002 in activation of macrophages in vitro.

Table 19.A. Tumor weight and incidence of metastases of L3.6pl human pancreatic tumors in nude mice after 4 weeks treatment with 100 mg/kg CPT-11 i.p. once a week +/- oral feeding of JBT 3002 (tablet) 0.05 mcg/dose

Treatment start with CPT11: 7 days after orthotopic tumor cell injection Treat

	uom uns	- CPT11
ານໄຂຕາດນ	sat	
ic tuillor ce	£	JBT3002
er ortnotop	wed thurs	JBT3002 JBT3002
313002: 3 days after orthotopic tuillor cell injection	wed	JBT3002
Treatment start with JB!	Treatment schedule:	

tues

(animals were sacrified 31 days after tumor cell injection)

П	Т							_				П		
		WT/PC	•	,	•	•	•	•	•	•	•	•	0/10	
		LN met	•	•	•	•	+	1	•	•	•	•	1/10	
	Incidence	liver met	•	ı	•	•	•	1	•	1	ı	•	0/10	
CPT11 + JBT 3002	Tumor weight (mg)		09	201	208	78	365	0	118	175	199	140	157.5 365 0	154.40
		WT/PC	•	•	•	•		•	,	•	•	•	0/10	
		LN met	++	‡	++		+	,	+	+	++	đ	7/10	
	Incidence	liver met		•	•	•	1	•	•	•	•	•	0/10	
CPT11	Tumor weight (mg)		08	375	241	0	86	0	318	137	205	29	117.5 375 0	152.10
	animal		•	. 2	l 69	4	ۍ د	9		. co	6	9	Median Max Min	Average

Table 19B. Tumor weight and incidence of metastses of L3.6pl human pancreatic tumors in nude mice after 4 weeks treatment with 100 mg/kg CPT-11 i.p. once a week +/- oral feeding of JBT 3002 (tablet) 0.05 mcg/dose

Treatment start with JBT3002: 3 days after orthotopic tumor cell injection Treatment start with CPT11: 7 days after orthotopic tumor cell injection

CPT11 mon sun sat JBT3002 JBT3002 JBT3002 thurs wed Treatment schedule:

tues

(animals were sacrified 31 days after tumor cell injection)

	Control (HBSS)				JBT-3002			
animal	Tumor weight (mg)	Incidence			Tumor weight (mg)	Incidence		
		liver met	LN met	WT/PC		liver met	LN met	WT/PC
•	534	,	‡	•	862	•	++	M
۰ ،	556	•	++	WT/PC	871	1	+	•
٦ ،	483	,	++	•	981	+ (5)	‡	W
> ∀	831	+ (3)	++	•	621	,	‡	M
٠ ،	955	+ (5)	+	.•	362	•	+	•
. · ·	73	+	‡	•	733		‡	•
· ·	578		‡	•	559	ı	•	•
- α	723	+ (1)	+++		820	+ (1)	+	•
· σ	701		++	ΤW	547	•	•	•
, 은		ı	++	WT			•	٠
Median	578	4/10	10/10	3/10	733	2/9	7/10	3/10
Max	955				981			
Min	73				362			
Average	603.78	·			706.22			
St.Dev.					163.53			

Table 19C. Tumor weight and incidence of metastases of L3.6pl human pancreatic tumors in nude mice after 4 weeks treatment with 100 mg/kg CPT-11 i.p. once a week +/- oral feeding of JBT 3002 (tablet) 0.05 mdg/dose

Treatment start with JBT3002: 3 days after orthotopic tumor cell injection Treatment start with CPT11: 7 days after orthotopic tumor cell injection

tues CPT11 mon sun sat JBT3002 JBT3002 JBT3002 Ē thurs wed Treatment schedule:

(animals were sacrified 31 days after tumor cell injection)

	tumor weight in mg	Incidence	
therapy	median (range)	liver met.	LN met.
Control (HBSS)	578 (73 - 955)	4/10	10/10
JBT3002	733 (362 - 981)	2/9	7/10
CPT11	117.5 (0 - 375)	0/10	7/10
CPT11+JBT3002	157.5 (0 - 365)	0/10	1/10

THEFT. THEIRTY OF EXPERIMENTAL HISTORIES PRODUCE BY ANIALONA MEMBER SOLVE SELECTION TO SELECT OF THE PROPERTY.

oral JBT 3002 in nude mice

									· .	
TWRFSS					TWRFSS		υ	b b	ט ט י	
W R F S S M					 WRFSSM		C 5604: 75	p.	J C, J 566% 71	05. 7082
R F S S M T	·	ບ ບ ບ		ນ ນ ນ	RFSSMT		(52) D	p.	ט ה (ינל) ט	oral
7/27 M T W			p p	p p	MTM			b b	p p	ose)
Intensive M	#5594 #5595 T	#5596 #5597 T	#5598 #5599 T	#5600 #5601 T	Once a week M	#5602 T #5603	#5604 #5605	#5606 #5607	#5608 #5609	T: KM12sm 1x10°6 i.spl J: FF-JBT3002 (0.05mcg/d C: CPT-11 (50mg/kg) i.p.

H. SHINOHARA July 22,1998

Table 21. Therapy of experimental liver metastases produced by CT-26 murine colon carcinoma with CPT-11 i.p. plus oral

JBT 3002 (free-form or tablet) in BALB/c mice

	41/2			
		7 7 8 8 8 M T	NR FSSMTWR	21 F S S M
INTENSIVE TREATMENT	; ;)			
Group I (n=5) Control 7332 II (n=5) CPT-11 7332 III (n=5) FF-JBT 7332 IV (n=5) TAB-JBT 7255 V (n=5) FF-JBT/CPT-11 7235 VI (n=5) TAB-JBT/CPT-11 7232	6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6	0 0 0 0 0 0 0 0 0		
ONCE A WEEK TREATMENT				
Group I (n=5) Control 753 ? II (n=5) CPT-11 753 ? III (n=5) FF-JBT 734 0 IV (n=5) TAB-JBT 754 ! V (n=5) FF-JBT/CPT-11 754 ? VI (n=5) TAB-JBT/CPT-11 754 ?	6 6 6 6 6 6 5 5 5 5	0 00 0 00	0 00 0 00 0 0 0 0 0 0 0 0 0	ပ ပပဲ
Legend T: CT26, 10,000 cells, i.spl (by Shinohara and Ozawa) C: CPT-11, 100 mg/kg, i.p. (by Shinohara and Ozawa) J: JBT 3002 (free form or tablet solution), 0.05 mcg/dose,	(by Shinohara and Ozawa) (by Shinohara and Ozawa) et solution), 0.05 mcg/d	wa) .wa) g/dose, oral	(by Jerry)	

H. SHINAHARA Aug. 6, 1998